

Chromium in urothelial carcinoma of the bladder

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Abstract

Introduction and objectives. Many epidemiological and experimental studies report a strong role of chemical carcinogens in the etiology of bladder cancer. However, the involvement of heavy metals in tumorigenesis of urothelial carcinoma of the bladder has been poorly investigated. Therefore, the aim of this study was to examine the relationship between chromium (Cr) and bladder cancer.

Materials and methods. Chromium concentration in two 36-sample series of bladder cancer tissue and sera from patients with this neoplasm were matched with those of a control group. The amount of trace elements in every tissue sample was determined using atomic absorption spectrometry. This was correlated with tumour stage.

Results. While the median chromium concentration levels reached statistically higher values in the bladder cancer tissue, compared with the non-cancer tissue (99.632ng/g and 33.144ng/g, respectively; $p < 0.001$), the median Cr levels in the sera of the patients with this carcinoma showed no statistical difference when compared to those of the control group (0.511μg/l and 0.710μg/l, respectively; $p = 0.408$). The median levels of Cr in the bladder tissue, depending on the stage of the tumour, compared with the tissue without the neoplasm, observed the same relationship for both non-muscle invasive and muscle-invasive tumours ($p < 0.001$ and $p < 0.01$, respectively).

Conclusions. This study shows that patients with urothelial carcinoma of the bladder had higher tissue Cr levels than people without tumour, while no difference was found in the Cr serum levels between the two groups of patients under investigation.

Key words

chromium, bladder cancer, heavy metals

INTRODUCTION

There is an emerging understanding of toxic and carcinogenic effects of certain heavy metals and trace elements in both animals and humans [1–7]. Chromium (Cr) is a known human carcinogen and has been hypothesized as a cause of several cancers, including leukaemia and neoplasms of lung, nose and nasal sinuses [8–10]. Moreover, there is epidemiological evidence suggesting that Cr is a plausible bladder carcinogen [11–14]. Additionally, higher Cr levels in the urine of people occupationally exposed to this element have been reported, further suggesting its possible contribution to lower urinary tract carcinogenesis [15]. Thus far, however, there has been no demonstrable direct evidence for the association of Cr with human urothelial carcinoma of the bladder.

In the presented study, the concentration of chromium in the tissue of bladder tumour was measured and compared with the content in non-cancerous tissue. In addition, the concentration of Cr in the sera of patients with bladder tumours were compared with control samples from patients with non-neoplasm- or non-heavy metal-related diseases of the urinary system. Finally, the resultant levels were evaluated, depending on the stage of the tumour.

MATERIALS AND METHOD

The study was approved by the Medical Ethics Committee for Human Studies of the Medical University in Bialystok, Poland, and all procedures were performed in accordance with the Helsinki Declaration of 1975, as revised in 1983. The study was conducted on 36 patients with histologically-proven urothelial bladder carcinoma, with no lymph node involvement or distant metastases. The patients were 30 men aged 41–88 (average 68.5 years) and 6 women aged 52–78 (average 67 years). Non-muscle invasive cancers (Tis – Ta – T1) occurred in 22 cases, and muscle-invasive tumours (T2 – T3 – T4) in 14 patients. The grade of histological malignancy, G1, G2 and G3, was found in 10, 13, and 10 patients, respectively, whereas in 3 patients it was not possible to establish with certainty the actual grade of malignancy.

The tumours were removed by transurethral resection (TURBT) in 29 patients and by cystectomy in 7 patients. Just after the bladder or the tumour itself had been removed, samples of 1g of cancer tissue were taken and stored after being snap frozen in liquid nitrogen. Before bladder or tumour removal, three-milliliter samples of venous blood were collected from the basilic vein using Vacutainer vacuum sets.

The control material consisted of 15 one-gram samples of bladder tissue taken from gender-and-age-matched individuals who had died from trauma ($n = 15$). Samples were taken during autopsy from 12 male cadavers aged 54–77

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(average 64 years) and 3 females aged 57–76 (average 65.5 years).

Next, 15 three-millilitre samples of venous blood from the corresponding gender-and-age-matched patients, who were in fasting states with non-neoplasm or trace elements-related diseases of the urinary system (1 woman with benign pelvic-ureteric junction obstruction, 3 with stress incontinence, 3 men with scrotal injury, 3 with renal injury, 2 with benign urethral stricture and 3 with hydrocele), were collected in Vacutainer vacuum sets and used in the control group. There were 11 samples taken from male patients aged 43–86 (average 67), and 4 from females aged 52–78 (average 67.5 years). There was no difference between the socio-economic status of the cases and controls. Neither group had occupational exposure to chromium.

Before analysis, the samples were mineralized in concentrated nitric acid (V) in a UniClever closed microwave system manufactured by Plazmatronika. The mineralization products were quantitatively transferred into polypropylene scintillation vessels. Cr was quantitatively determined directly in the sera samples diluted with 0,2% Triton X-100. The levels of Cr concentration were analyzed by the atomic absorption spectrometry technique with the Zeeman background correction on a Hitachi Z-5000 spectrometer. The Cr content was calculated using readings on a standardisation curve formed by recording differences in absorbance and element concentration. The accuracy of the Cr determination method was verified using the following certified standard materials: Seronorm 404108 for the whole blood and BCR 184 for bovine muscle. Accuracy (i.e. % of error) and coefficient of variation were calculated for the certified standards under investigation. The samples were evaluated in the Department of Bromatology of the Medical University in Bialystok, which is involved in a programme of intra-laboratory comparative analysis of elements, organized by the Institute of Chemistry and Nuclear Techniques, and the State Institution of Sanitation in Poland.

Since data in the studied group was not distributed in a Gaussian manner, the Mann-Whitney U test was used. A p value of < 0.05 was considered statistically significant. The Statistical Package for the Social Sciences (SPSS) was used for all statistical analyses.

RESULTS

Median values of Cr, determined in the bladder cancer tissue and sera of the patients with bladder tumour and the control group, are shown in Table 1. Figures 1–2 present Cr bladder tissue and serum concentration distribution of patients with bladder cancer and controls.

Table 1. Bladder tissue and serum chromium concentrations in patients with bladder cancer vs. controls

Group		n	Q1	Median	Q3	p
Cr serum, µg/l	bladder cancer cases	36	0.353	0.511	0.920	0.408
	controls	15	0.388	0.710	0.942	
Cr bladder tissue, ng/g	bladder cancer cases	36	51.045	99.632	186.582	0.001*
	controls	15	24.764	33.144	42.605	

Cr – chromium; * – statistically significant; n – number of samples; Q1 – lower quartile; Q3 – upper quartile

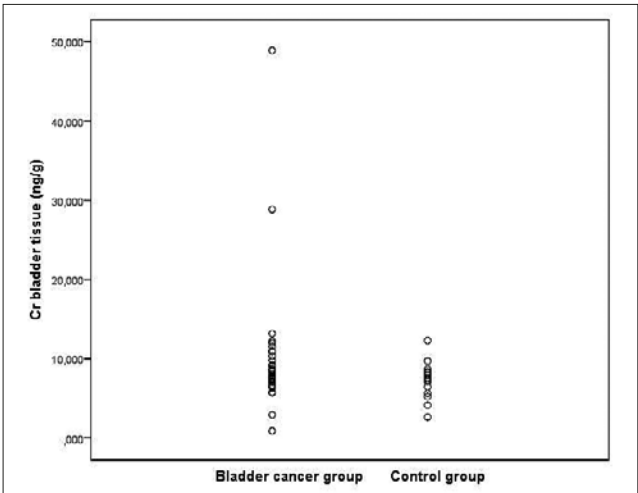


Figure 1. Chromium (Cr) distribution in bladder tissue of patients with bladder cancer and control group

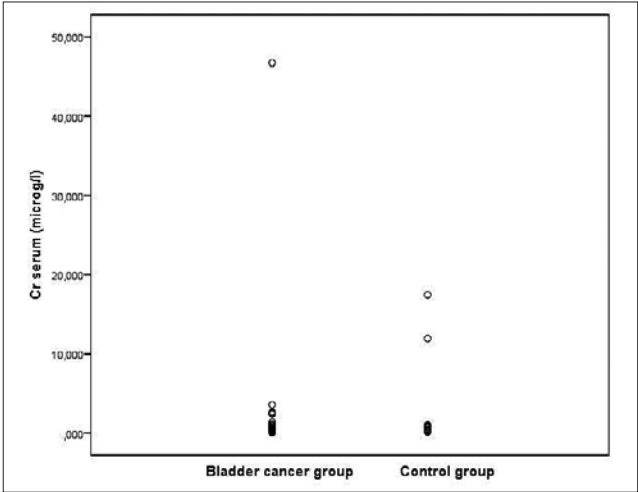


Figure 2. Chromium (Cr) distribution in sera of patients with bladder cancer and control group

The median value of Cr concentration in the bladder cancer tissue and in the non-cancer tissue was 99.632ng/g and 33.144ng/g, respectively. This increase in Cr concentration in cancer tissue was statistically significant ($p<0.001$). There was no significant difference in the serum Cr levels between the groups (0.511µg/l and 0.710µg/l, respectively; $p=0.408$). Moreover, tissue and serum Cr levels were calculated for the two tumour stage groups, (one with non-muscle invasive and the other with muscle invasive cancers). When processed by the non-parametric Mann-Whitney U test, the results showed that the median levels of Cr in the bladder tissue, depending on the stage of the tumour, were significantly higher for both non-muscle invasive and muscle-invasive tumours, compared with the tissue without the neoplasm ($p<0.001$ and $p<0.01$, respectively). There was no marked difference in the Cr serum levels, between people with urothelial carcinoma of the bladder and the controls when analysed by the two tumour stage groups (Tab. 2).

Table 2. Bladder tissue and serum chromium concentrations in patients with non-muscle invasive vs. muscle invasive bladder cancer

Group	n	Q1	Median	Q3	p (vs. control)
Cr serum, µg/l	Tis+Ta+T1	22	0.375	0.522	0.680
	T2+T3+T4	14	0.345	0.411	0.270
	controls	15	0.388	0.710	
Cr bladder tissue, ng/g	Tis+Ta+T1	22	63.785	106.28	0.001*
	T2+T3+T4	14	27.661	69.184	0.01*
	controls	15	24.764	33.144	

Cr – chromium; n – number of samples; Q1 – lower quartile; Q3 – upper quartile; vs. – versus; * – statistically significant

DISCUSSION

In the study, a statistically significant elevation of tissue chromium levels was observed in patients with bladder cancer, and no difference in the serum Cr levels between the two groups of patients under investigation. To the best of the authors' knowledge, there has been no previous report on Cr concentration levels in the bladder tissue and sera of patients with bladder cancer and controls. However, several epidemiological studies have suggested an association between Cr exposure and the risk of urinary bladder cancer [11–14]. Kutze et al. in their study, found a significantly increased probability of developing lower urinary tract urothelial tumours in patients occupationally exposed to chromium/chromate [13]. The observed relationship was confirmed in a multivariate logistic regression analysis, which showed a significant contribution of Cr to the risk of bladder cancer. These observations were further corroborated by the findings from the matched case-control study of 431 patients with urothelial cancer of the lower urinary tract from Northern Germany [12]. In this report, occupational exposure to chromium/chromate was associated with the increased risk of BC (odds ratio of 1.77; 95 per cent confidence interval =1.06–2.97). Interesting data is provided by Stamatiou et al. who followed 45 patients with non-muscle invasive bladder cancer for a period of three years [14]. The authors observed an exceptionally high recurrence rate of BC (42%) in their sample, which occurred within a three months interval. Although they did not directly assessed the levels of Cr in the patients' tissues, the authors hypothesized that the reported unusually frequent reappearance of bladder cancer in that group of patients could have been associated with the consumption of contaminated tap water containing large amounts of hexavalent chromium.

The aforementioned observations linking Cr to bladder cancer could be related to the fact that the urinary system is very much involved in the Cr removal process from the human organism, as excretion of this element occurs predominantly through urine [16]. Thus, whenever this element is present in urine, it may directly act on the urothelium, especially inside the bladder, which functions as a temporary reservoir for urine and, as such, is exposed to Cr action for a longer time. Of added interest is evidence suggesting that urinary Cr levels increase with time of occupational exposure, which potentially could increase the risk for developing a bladder neoplasm [15, 17].

The International Agency for Research on Cancers has classified Cr (VI) as Group I (human carcinogen), whereas Cr (III) as Group III (non-carcinogenic to human) [8]. Similarly, The United States Environment Protection Agency (EPA) has classified Cr in +6 oxidation state as Group A, a known human carcinogen, and Cr in +3 oxidation state as Group D (non-carcinogenic to humans) [18]. The mechanisms involved in chromium-induced carcinogenesis are not, however, completely understood. Depending on the oxidation state of the chromium and presence of cellular reductants, it causes a wide variety of DNA lesions including Cr-DNA adducts, DNA-protein crosslinks, DNA-DNA-crosslinks, lipid peroxidation and oxidative damage [19, 20]. Although evidence for Cr(VI)-induced carcinogenicity and mutagenicity has been well recognised, the capability of Cr(III), and in other oxidation states to produce free radicals in the presence of certain ligands, have been learnt only recently [21–24].

The exact pathological mechanisms for alterations in Cr tissue levels in patients with bladder carcinoma remain unclear. For the time being, it is difficult to say whether observed differences in levels of this heavy metal in tissues are the causative factors or the results of the neoplastic process. However, in the presented study, occupational exposure was ruled out as a cause for element alterations in both the BC patients and controls.

The current study found no difference in the serum Cr levels between the two groups of patients under investigation. This observation can be attributed to the fact that serum Cr concentration reflects current exposure rather than whole-body burdens [15]. Therefore, absence of difference in Cr serum levels between subjects with bladder cancer and controls provides an indirect evidence that both groups of people had similar exposure to chromium.

North-Eastern Poland is regarded as an unpolluted region and has been found to have low environmental contamination with Cr [25]. This is mainly due to a lack of anthropogenic sources of heavy metals, such as industrial waste, mining or smelting activity. Automobile exhausts, municipal effluents and smoking are the major sources of Cr in this area.

CONCLUSIONS

Patients with urothelial carcinoma of the urinary bladder show alterations in the Cr levels in tissue suggesting an association between this cancer and the heavy metal. Higher Cr levels may play a role in the induction and development of the bladder tumour, or may be the result of the neoplastic process. Further studies should assess the biological significance of these parameters and their relationship with other contributing neoplastic factors.

Conflict of interest

The authors declare that they have no conflict of interest.

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